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Neurotrophins play differential roles in short and long-term recognition memory

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ABSTRACT

The neurotrophin family of proteins are believed to mediate various forms of synaptic plasticity in the adult brain. Here we have assessed the roles of these proteins in object recognition memory in the rat, using icv infusions of function-blocking antibodies or the tyrosine kinase antagonist, tyrphostin AG879, to block Trk receptors. We report that tyrphostin AG879 impairs both short-term and long-term recognition memory, indicating a requirement for Trk receptor activation in both processes. The effect of inhibition of each of the neurotrophins with activity-blocking neutralising antibodies was also tested. Treatment with anti-BDNF, anti-NGF or anti-NT4 had no effect on short-term memory, but blocked long-term recognition memory. Treatment with anti-NT3 had no effect on either process. We also assessed changes in expression of neurotrophins and their respective receptors in the hippocampus, dentate gyrus and perirhinal cortex over a 24 h period following training in the object recognition task. We observed time-dependent changes in expression of the Trk receptors and their ligands in the dentate gyrus and perirhinal cortex. The data are consistent with a pivotal role for neurotrophic factors in the expression of recognition memory.

1. Introduction

Acquisition and consolidation of newly-acquired information are neuroplastic processes that may share common signaling pathways. Memory consolidation is commonly viewed to be a process lasting several hours through which memories are transformed from a labile to a more stable state (Bliss & Collingridge, 1993; Izquierdo et al., 2006; Kandel, 2001; Riedel, Platt, & Micheau, 2003). Functional integrity of the medial temporal lobe including the hippocampus proper, the dentate gyrus and parahippocamal cortices are essential for object recognition memory processing (Brown & Aggleton, 2001; Clarke, 2000; de Lima, Luft, Roesler, & Schroder, 2006; Ennaceur & Delacour, 1988; Malleret et al., 2010; Myskiw et al., 2008). However, much debate surrounds the relative importance and differential input of the perirhinal cortex and the hippocampus (Brown & Aggleton, 2001). It has been proposed that the perirhinal cortex is critically involved in discrimination of familiarity, whereas the hippocampus appears to support contextual memory, but may not be necessary for familiarity discrimination (Balderas et al., 2008; Brown & Aggleton, 2001; Ennaceur, Neave, & Aggleton, 1996; Gaskin et al., 2010; Lehmann, Glenn, & Mumby, 2007; Mumby, Tremblay, Lecluse, & Lehmann, 2005; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). Data from our laboratory indicate that BDNF-stimulated signaling pathways in both dentate gyrus and perirhinal cortex contribute to long-term recognition memory (Callaghan & Kelly, 2012).

The cellular and molecular signaling cascades that contribute to short- and long-term recognition memory have yet to be fully elucidated. Learning-induced synthesis of new proteins is believed to play a pivotal role in the consolidation process (Rossato et al., 2007; Winters, Tucci, Jacklin, Reid, & Newsome, 2011) but the specific proteins underpinning these events and timing of their translation remain unidentified. However, a clear role for neurotrophins in various forms of synaptic plasticity, including expression of long-term potentiation (LTP) and memory acquisition and consolidation, has been established (Alonso et al., 2005; Bekinschtein et al., 2007; Bramham, Southard, Sarvey, Herkenham, & Brady, 1996; Chen, Kitanishi, Ikeda, Matsuki, & Yamada, 2007; Gooney & Lynch, 2001; Griffin, Bechara, Birch, & Kelly, 2009; Hennigan, Callaghan, Kealy, Rouine, & Kelly, 2009; O'Callaghan et al., 2009; Rattiner, Davis, French, & Ressler, 2004). Much of this evidence supports a specific role for the TrkA ligand NGF and the TrkB ligands BDNF and (to a lesser extent) NT4, in expression of LTP and learning and memory. Less evidence exists to support similar functions for NT3; indeed decreased NT3 expression has been observed in responce to LTP or seizure activity (Castren et al., 1993; Lindholm, da Penha Berzaghi, Cooper, Thoenen, & Castren, 1994; Lindvall, Kokaia, Bengzon, Elmer, & Kokaia, 1994), while NT3 has also been linked to perforant path paired pulse depression (Asztely, Kokaia, Olofsdotter, Ortegren, & Lindvall, 2000).

In the context of recognition memory, Seoane, Tinsley, and Brown (2011) have recently shown that interference with BDNF

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in the perirhinal cortex blocks long-term recogniton memory but similar studies investigating the potential roles of NGF, NT3 or NT4 in recognition memory are lacking. Here we aimed to investigate the roles of each of the neurotrophin family of proteins expressed in mammalian cells in both short- and long-term recognition memory. To do so, we used neutralising antibodies against each neurotrophin and targetted the Trk receptor using the tyrosine kinase antagonist tyrphostin AG879 (Eisinger & Ammer, 2008; Maguire, Casey, Kelly, Mullany, & Lynch, 1999; Zhang, Chi, & Nicol, 2008). We also assessed whether the consolidation of recognition memory over a 24 h period is associated with changes in expression of neurotrophins and their receptors in relevant structures of the medial temporal lobe.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–350 g, total number = 78) were obtained from the BioResources Unit (BRU) of Trinity College Dublin. They were housed in groups of four, were provided with food and water *ad libitum* and experienced a 12 h light:dark cycle in a temperature-controlled environment (20–22 °C). Experiments were conducted under national law and European Union directives on animal experiments.

2.2. Surgical procedure and drug delivery

Rats (animals for surgery = 42) were anesthetized with ketamine (100 mg/kg, i.p.; Bayer Healthcare) and xylazine (100 mg/kg, i.p.: Rompun®, Bayer Healthcare) and supplemented throughout the surgical procedure as necessary. A single hole was drilled in the skull over the left ventricle (coordinates, bregma, 0.9 mm; midline, 1.3 mm) and a cannula (Bilaney Consultants Ltd., Kent, UK) was lowered slowly into the ventricle to a depth of 3.6 mm below the brain surface. A guide cannula (Plastics 1, Bilaney Consultants Ltd., Kent, UK) was held in place and fixed to the skull with dental cement (Prestige Dental Products, West Yorkshire, UK). The incision was closed with surgical staples (Promed, Ireland). Rats recovered for 7–10 days before being tested.

2.3. Object recognition task

The apparatus consisted of a black circular open field (diameter, 1 m; height, 0.5 m) placed in a dimly-lit room. Rats were handled daily for one week and habituated to the experimental apparatus by 20 min of exploration in the absence of objects each day for three days before the experiment was performed. Habituation was deemed to be successful if animals actively explored the open field. Objects were constructed from toy bricks and fixed to the floor of the open field 15 cm from the walls. Objects were cleaned thoroughly between trials to ensure the absence of olfactory cues. The criteria for exploration were strictly based on active exploration, in which rats had to be touching the object with at least their noses. Measurement of the time spent exploring each object was recorded and expressed as a ratio of the total exploration time in seconds. Rats were placed into the arena at random entry points for 3 sample phases (5 min each with an inter-trial rest period of 5 min; trial 1, trial 2 and trial 3 respectively). In the sample phases animals were exposed to objects A and B and the time spent exploring Object A and B was measured using stopwatches. The protocol involved two choice phases where a familiar object was exchanged for a novel object (C or D) placed in exactly the same position. The first choice phase was at 10 min (Trial 4; 5 min; Choice phase 1 (T1)) following sample phases and the second choice phase was at 24 h (Trial 5; 5 min; Choice phase 2 (T2)) following sample phases. Time spent actively exploring each object during choice phases was recorded and calculated as a ratio of the total exploration time. In some experiments, cannula-implanted rats were administered an injection, through the internal cannula that was fitted to the guide cannula, with the tip of the internal cannula protruding 1 mm beyond the guide cannula, insuring the treatment reaches the ventricle directly, in agreement with the atlas of Paxinos and Watson (1998). Infusions of anti-BDNF, anti-NGF, anti-NT3, anti-NT4 or sheep serum or rabbit serum (5 μl; Chemicon) or Tyrphostin AG879 (10 μl; 0.5 mM in DMSO 10% (v/v) in PBS; Calbiochem) or vehicle control (DMSO 10% (v/v) in PBS) were performed over 3 min, the needle was kept in place for a further 1 min to prevent back-flow, 60 min prior to the training phase. The biological effect of IgG1 antibodies is reported as 11 day half-life in the literature (Davis et al., 1995), and according to manufacturer anti-BDNF, anti-NGF and NT4 has less than 1% cross reactivity and anti-NT3 has less than 0.1% cross reactivity with other neurotrophins when measured by ELISA or dot blot analysis. In some experiments, animals underwent the training phase only and were sacrificed at 0 h, 2 h, 6 h or 24 h following training. Two control groups were included, a group of naive animals were sacrificed directly from the home cage and a group of control animals were habituated to the open field in the absence of objects for 3 × 5 min trials with an inter-trial interval of 5 min.

2.4. Sample preparation

Rats were killed by cervical dislocation and decapitation; brains were removed immediately and the hippocampi, dentate gyrii and perirhinal cortices were dissected free on ice. A small piece of each sample was was placed in RNAlater (200 μl) and stored at 4 °C, snap frozen with liquid nitrogen within seven days, and stored at −80 °C for later analysis by RT-PCR. The remainder of the tissue samples were sliced and stored according to the method of Haan and Bowen (1981). Tissue samples were sliced bi-directionally to a thickness of 350 μm using a McIlwain tissue chopper and rinsed twice in ice-cold oxygenated Krebs solution (NaCl, 136 mM; KCl, 2.54 mM; KH_2PO4, 1.18 mM; $Mg_2SO_4{\cdot}7H_2O$, 1.18 mM; $NaHCO_3$, 16 mM; Glucose, 10 mM; $CaCl_2$, 2 mM, protease inhibitor cocktail 1 and 2 (Sigma UK) 1:100 dilution, phosphatase inhibitor cocktail (Sigma UK) 1:100 dilution), twice in with ice-cold oxygenated Krebs solution containing DMSO (final concentration: 10%) and stored in this solution at −80 °C. When required, slices were thawed rapidly at 37 °C and washed three times with ice-cold oxygenated Krebs solution and homogenized in the appropriate buffer.

2.5. Analysis of NGF, BDNF, NT3 and NT4 concentration by ELISA

Neurotrophin expression was analyzed in supernatant prepared from homogenate of dentate gyrus, hippocampus and perirhinal cortex. Tissue slices were homogenized in Krebs buffer (350 μl) and centrifuged at 1400 rpm for five min; supernatants were removed and analysed by ELISA. The concentrations of NGF and NT4 were quantified by ELISA (R&D Systems) according to the manufacturer's instructions. Briefly, 96-well plates (MaxiSorp; NUNC) were coated overnight at room temperature with anti-NGF or anti-NT4/5 antibody (100 μl; diluted in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4; pH 7.2); 1:180 in the case of NGF; 1:360 in the case of NT4). The plates were washed 3 times in PBS-T (300 μl; 0.05%Tween-20 in PBS) using an automated plate washer and blocked with block buffer (300 μl; bovine serum albumin (BSA, 1% (v/v)) for 1 h at room temperature. The plates were subsequently incubated with samples and serially-diluted NGF or NT4 standards (50 μl) for 2 h at room temperature, washed, and incubated with anti-NGF or anti-NT4 detection

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